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Art. II.—*Outbreaks of White Spot or Ichthyophthiriasis* (Ichthyophthirius multifiliis Fouquet, 1876) *at the Hatcheries of the Ballarat Fish Acclimatization Society with notes on Laboratory Experiments.*

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Contents.

Introduction.

This work has been carried out in my capacity as Biologist on the Victorian Fresh Water Research Committee. This Committee, which is an honorary one, conducts investigations into fresh water problems throughout the State. White Spot is one of the varied problems which have been studied in the three years of the Committee's existence. My own work was carried out in the Zoology Department, University of Melbourne, and two visits were made to the hatcheries at Ballarat .

The parasite responsible for this disease has been introduced into Australia and one certain source is from imported ornamental carp. It is impossible to state the first date of entry; only in recent years has the disease been of any great economic importance. Previous to that it had been observed in private aquaria over a considerable period, frequently causing the death of small exotic fish.

Outbreaks of this disease, on an epidemic scale, occurred at the hatcheries late in the 1938-39 season when yearling trout were infected, and again early in the 1939-40 season when trout fry were infected. The first outbreak occurred on 3rd April, 1939, and the second was reported on 21st November, after having appeared some days earlier. Both outbreaks showed up very suddenly and were severe in their effect. The losses for the two seasons are set out in Table I.

TABLE I.—Losses caused by White Spot.

	Brown Trout.	Rainbow Trout.	Total Losses.
1938–39	50,000	30,000	80,000
1939–40	275,000	95,000	370,000

The parasite population probably was building up prior to the recognition of the disease and under suitable conditions reached epidemic proportions.

This report refers in particular to the second outbreak although mention will be made of the first when it differs in some respect from the second.

Occurrence of the Disease.

Serious epidemics have been reported from time to time in France, Holland, Germany, and in various parts of the United States of America. The disease was first recorded in 1869 in the Zoological Garden in Hamburg, Germany, and in France in 1876, where careful observations were made and the name *Ichthyophthirius multifiliis* was proposed by Fouquet for the pathogenic organism—(Prytherch 1924).

In October, 1929, an epidemic at the Ballarat hatcheries was reported to be White Spot but the description of the symptoms in the Minutes of the Society does not warrant such a conclusion. The outbreak was not investigated on that occasion.

An outbreak of White Spot occurred at the Plenty hatcheries, Tasmania, in February, 1933, when trout fry and yearlings died. In a report on the outbreak by the Honorary Pathologist of the Tasmanian Salmon and Freshwater Fisheries Commission it is stated that few, if any, of the deaths were caused by the parasite. The probable cause was that just prior to the outbreak the sediment at the bottom of the ponds had been stirred up by dragging bluestone bags over the bottom in an attempt to get rid of a heavy growth of weed—(Salmon and Freshwater Fisheries Commission (Tasmania). Report, June, 1933).

In July, 1933, White Spot was reported on young blackfish (*Gadopsis marmoratus*) in a private pond at Heidelberg (Melbourne). This diagnosis was later confirmed.

Numerous reports have been received of cases of White Spot on aquarium fish in Victoria and New South Wales.

The disease is also prevalent in Japan. It attacks only fish which are confined to ponds or aquaria, and is never found on those living in lakes and rivers—(Roughley, 1933).

In August, 1937, the disease was reported from Capetown, South Africa. It had been introduced with imported tropical aquarium fish. An Honorary Fishing Inspector succeeded in infecting a small indigenous fish (*Spirobranchus capensis*) with parasites from imported fish—(Cape Piscatorial Society, South Africa, 1937).

Life Cycle of the Parasite—Conditions Favouring an Outbreak of the Disease.

White Spot is caused by a ciliate protozoan parasite which is whitish in colour and visible macroscopically; the size ranges from one-half to slightly less than one millimetre in diameter. The body is round or elongate oval in shape and movement is by an active rotation brought about by the action of the cilia which are arranged regularly in rows. "The ciliate fixes itself to the skin and gradually becomes embedded in the epidermis." (Wenyon, 1926.)

The life cycle is complicated but for all practical purposes it may be divided into four phases (Fig. 1).

The disease derives its name from the appearance of the infected fish. The skin may be raised into pustules which are whitish in colour (hence the name "White Spot") over any part of the fish, body, head, fins, tail, gills, etc., in a young form, and over the gills, fins, tail or on any unscaled portion in an older individual. Within the pustules are the parasites and they remain there for a period of four to nine days or longer, the period being determined by the water temperature. The parasite passes its growing phase in this position. Numerous transverse sections have been taken through the pustule and the body wall of the fish, and although there is no doubt that the parasite does increase in size within the pustule, there is no visible evidence of any breakdown of the tissues or of the skin within which the parasites lie. Roughley (1933) states that "Occasionally these blisters are found to contain several of the parasites, which have resulted from the division of the original one into several parts." But after examining in stained preparations several hundred pustules containing very numerous individual parasites this statement cannot be supported. According to Wenyon (1926) "There is some doubt as to whether multiplication of the ciliate can take place within the pustules, for the presence of two parasites in a single pustule, as sometimes occurs, may be merely an indication of an invasion of one spot on the skin by two ciliates." In all the material examined from Ballarat the pustules have contained from several up to 40 or more individuals and the occurrence of one or two individuals in a pustule has been rare. The parasite does not lose its locomotory power within the pustule; it may be observed under a microscope to be slowly rotating if

the skin bearing the pustules is stripped from an infected fish which has just been killed. When the pustule bursts the free-swimming forms are liberated into the water; these forms may be called the *Precystic* forms and they constitute the second phase of the life cycle. Experiments have shown that these forms do not reinfect a fish, but after a few hours in the free-swimming state they settle to the bottom of the pond and there encyst; this is the third phase. The process of encystment consists of the secretion of a gelatinous membrane followed by binary fission of the individual. The number of individuals

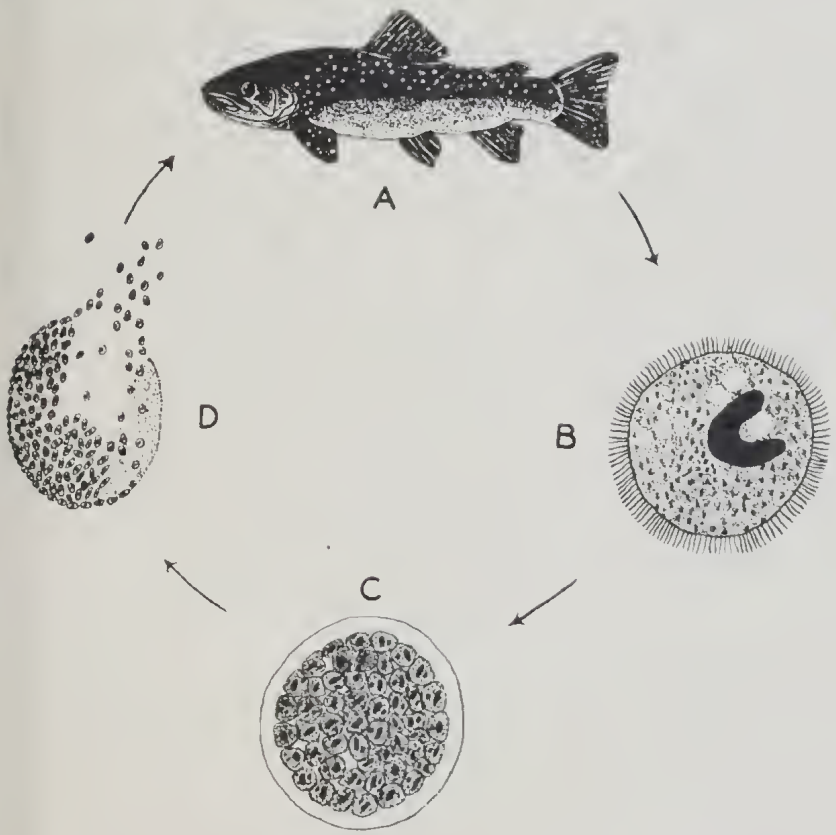

FIG. 1.—Diagrammatic representation of the life cycle of the parasite. *A*, adult parasites in pustules on a trout; *B*, parasite after leaving fish as a free-swimming pre-cystic form and about to settle to the bottom; *C*, division of the adult into many smaller individuals after formation of cyst; *D*, bursting of cyst, releasing hundreds of minute post-cystic forms of the parasite, which in turn reinfect the fish. (After Prytherch, slightly modified.)

arising from the single parasite which formed the cyst varies. Usually there are several hundred but the number may reach 2,000. Within a few hours the cyst ruptures and the new individuals which may be called the *Postcystic* forms are liberated.

These are again free-swimming and constitute the fourth phase of the life cycle. These are the forms which re-infect the fish and give rise, after a few days, to the characteristic pustules.

Thus, in the life cycle of the parasite there are two free-swimming phases and two fixed phases. All control measures must be aimed at the parasite while in any of its three stages away from the fish; this will be discussed later on. It is usually stated that the parasite is away from the host for a period of 24–36 hours, the time varying according to the temperature. Recent work, which will be dealt with more fully in a later paper, has indicated that the parasite may live for several days after it has been liberated from the cyst if the host is not available. This fact has to be taken into consideration in all control work.

Conditions which favour an outbreak of White Spot are:— (1) High water temperatures, 60°F. or greater, and (2) poor water supply.

These conditions were fulfilled in the first outbreak. Drought conditions had persisted over a considerable period; the water supply to the ponds was poor and temperatures ranging from 58–63°F. were a constant feature. In the more recent outbreak conditions were somewhat different. The water temperatures were fairly high, 62–64°F., but the water supply was excellent, sufficient being available since the commencement of the season. It may be seen that conditions favouring an outbreak were not at an optimum, but as against this, in the second outbreak, the fish infected were fry, which are more suspectible to the parasite than are yearlings.

In hatcheries the conditions favouring an outbreak are easily attained, and it is noteworthy that from hatcheries have come all the reports of serious epidemics. To my knowledge none of any importance have been reported from lakes or rivers. "In nature, on the other hand, ichthyophthiriasis is seldom observed, probably owing to the fact that under natural conditions only a very small proportion of the young parasites succeed in establishing themselves on a fish, and consequently the infections produced are so slight that they are overlooked, and the fish is unharmed" (Minchin, 1912). The possibility of an epidemic in a lake is slight and in a river even less so; in these environments, apart from the reason given by Minchin, suitable conditions are less likely to be reached.

The disease was more rapid in its effect this season (1939-40) than in the last, the size of the fish in each case again being the probable explanation.

While in Ballarat I examined a bottle of water which had been obtained, before my arrival, by dropping the bottle into an infected pond. It contained a large number of the free-swimming forms indicating the density of the parasite population.

Another point of general interest is the fact that in both outbreaks of the disease there have been a few fish which have survived in the infected ponds. This may be an indication that a few fish attain immunity.

The Source of Infection.

The distribution of the infection in the ponds during the early part of the 1939-40 season was puzzling, and at first threw no light on the probable source. The ponds at the hatcheries are in series, that is, water flows through one pond to the next. Ponds 6, 5, and 4, which are in series (see Fig. 2), and ponds 7–15, also in series, were infected. Pond 7 was the first to show infection in the outbreaks of both seasons, and the disease appeared to be most rapid in its effect in this pond on both occasions. An overflow pipe runs from pond 7 to pond 6, thus interconnecting the two series and providing the explanation for the spread of the disease through them. Ponds 17–46 form a separate unit (all not included in figure). Ponds 25 and 32, coming in series in this unit, were also infected, whilst ponds coming before and after them, and receiving the same water supply, were not infected.

The following possible sources of infection were suggested, and were examined in turn:—1. The Gong Reservoir; 2. Lake Wendouree; 3. Some source in the hatchery itself.

1. The Gong Reservoir.

This reservoir is the normal source of water for the hatcheries, but there is no evidence of the parasite coming from it. Several reservoirs form Ballarat's water supply, but the water ultimately passes through the Gong. The reservoirs are heavily stocked with English perch, and the Gong was netted, several hundred small fish being obtained. These were examined and none showed any signs of infection. A few fish were also taken from the Kirk reservoir (which supplies the Gong) and these again were clean. Evidence obtained at the hatcheries indicates that the English perch is either non-susceptible or is infected only with difficulty by *Ichthyophthirius multifiliis*. A small fish was taken from a pond in which several thousand trout had died, and in which were still large numbers of free-swimming parasites, and yet it showed no signs of infection, and was in perfect condition. Prytherch (1924) after giving a considerable list of species of fish for which the parasite has proven fatal, states that the perch, among a few others which he names, is seldom killed by the disease or affected to any noticeable degree.

2. Lake Wendouree.

Water is pumped from the lake to the hatcheries when the reservoir supply is unsatisfactory. All the water leaving the hatcheries runs into the lake, the outlet and the pump-line inlet

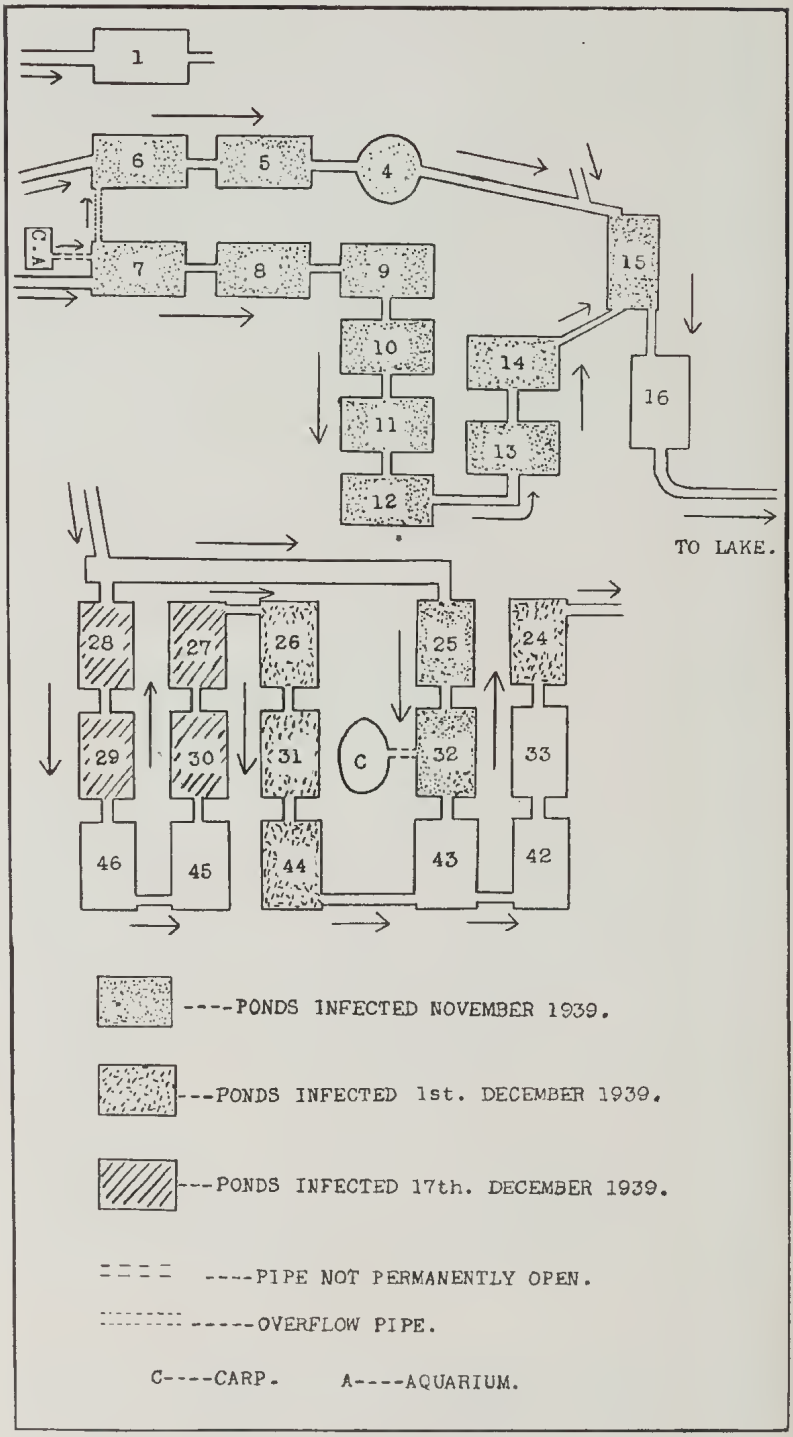

FIG. 2. Diagrammatic plan of the Ballarat hatcheries, showing only those ponds which are under discussion.

being normally some distance apart. During the 1938-39 season drought conditions prevailed and water had to be obtained from the lake. The lake level fell considerably, and to overcome this difficulty a large pit had to be dug from which water was pumped. Unfortunately, at the same time, in a further attempt to remedy the poor water supply, the outlet pipe was moved and all waste water was run into the pit. By this means water from the infected ponds ran into the pit and was carried back to the hatcheries. The water to the hatcheries first passes through concrete storage tanks before going to the ponds, and it is the usual practice to place fry in these. During the last season fish in one tank became infected. The lake must therefore have been the source of infection of this tank, even though the infection in the lake itself may have been derived from the hatcheries.

Apparently the parasite did not carry over the winter in the lake as lake water was used at one period early this season prior to the outbreak and the fish remained clean.

Unfortunately, again this season, infected water was run into the lake for two days without first being treated. Lake water has not been used since that date. Later, tests of the lake water and methods of treatment of the water running into the lake will be discussed. The inlet and outlet pipes from and to the lake are again some distance apart.

All evidence points to the fact that Lake Wendouree was infected from the hatcheries and that it is not the primary source of infection.

3. The Hatchery.

The third possible source of infection was from fish introduced into the hatchery itself. The only fish in the hatcheries were English perch, carp, and trout, both Rainbow and Brown. The perch, as already mentioned, are apparently not infected. A large aquarium of carp has been kept in the hatcheries for some years and this is only a few feet away from pond 7. These fish were examined, and in several cases were found to be carrying the parasites. The pustules were not numerous and no dead fish had been observed. Some time previous to the outbreak a tench had been placed in this aquarium and had been found dead some time later, but it was not examined. Prytherch (1924) states that ichthyophthiriasis is fatal to tench. The carp appear to act as carriers of *Ichthyophthirius multifiliis*. Once these fish were found to be infected all the evidence pointed to this aquarium being the source of infection. Pond 7 is in direct line with the aquarium and this is the pond which has been the first to show the infection on both occasions. Any heavy shower of rain will cause the aquarium to overflow into pond 7, and there is also a pipe connecting the pond and the aquarium, which has been used to empty the latter. Carp have been kept in the aquarium

for some years, but prior to the first outbreak of White Spot, fresh fish had been brought over from ornamental ponds in the adjoining Public Gardens. Japanese carp had been added to these ponds some time before this, and soon after some of these fish had been observed dead or dying. This fact had only been casually observed at the time and the fish had not been examined. As has already been mentioned, pond 6 is in direct communication with pond 7 by way of an overflow pipe, which explains how the second series became infected. Eighty thousand fry were lost early this season in these twelve ponds. Only a few carp from the ponds in the Gardens could be examined, and these showed no signs of infection. This evidence could not be taken as conclusive as these fish may not have been infected at the time, and larger numbers would have to be examined at intervals.

The explanation of the infection in ponds 25 and 32 is not quite so conclusive. Next to pond 32 (see fig. 2) is a small pond carrying carp. This carp pond is not in direct series with the main ponds, but is connected, by means of a pipe which is normally closed, to pond 32. The pipe had not been opened this season. Carp from here were examined but appeared clean. One dead fish floating on the surface of the pond was also examined, but although indications of infection were observed the fish had been dead too long to permit a definite diagnosis. The explanation of the infection in ponds 26, 31, and 44 later in the present season appears to be this: When the water level in a pond rises higher than normal, due to blockage of the outlet by silting of the wire screen across it, as occasionally occurs, the water may flow backwards through the inlet channel and thus enter other ponds. This is probably the explanation of the December 1st outbreak following on inefficient treatment of the ponds which were infected in the earlier part of the outbreak.

Although there is no direct evidence as to how the infection was carried to ponds 25 and 32 in the early part of the outbreak, there are several possible explanations.

The parasites may have been transferred by means of gear which had been used in infected ponds. This could have quite easily been done before there was any indication of the outbreak. The hatcheries are easily entered, and it is known that children interfere with the ponds in the absence of the hatcheries' staff. During one of my visits to Ballarat fish were found in ponds to which they could not have possibly moved by way of the water channels. It is possible that fry from the infected ponds could have been transferred to these ponds, which were clean in the early stages of the outbreak of disease. Birds bathe in the ponds, and the parasites may be transferred by this means. Rats also move from one pond to another. A further possible agent is the yabby (cray-fish) which is driven from the infected ponds by the salt treatment.

Control Measures.

The cheapest satisfactory means of control is by the use of sodium chloride. Laboratory tests have shown that sodium chloride and potassium permanganate destroy the parasite in its precystic, cystic and postcystic stages. The more detailed work was done with the precystic stage, using different concentrations of sodium chloride. Fifty tests were made, and the results of a number of these are given in Table II.

TABLE II.—Toxicity tests with sodium chloride.

Test.	Concentration of NaCl.	Number of Parasites Used.	Temperature.	Time of Cessation of Movement	Remarks.
	Per cent.				
1	0·5	3	16°3 C.	.	Still alive after 35 minutes
2	0·5	2	16°3 C.	.	
3	1·0	3	16°3 C.	.	Actively swimming for 3–5 minutes, then movement ceased. Recovered within half an hour
4	1·0	3	16°3 C.	.	
5	1·0	6	16°3 C.	.	
6	1·0	1	18°0 C.	.	
7	1·5	1	18°0 C.	2 min. 10 sec.	No recovery
8	1·5	1	18°0 C.	4 min. 20 sec.	
9	2·0	1	18°0 C.	1 min. 50 sec.	
10	2·0	1	18°0 C.	1 min. 40 sec.	Observations made 30 minutes later No recovery.
11	2·0	1	18°0 C.	1 min. 45 sec.	
12	2·0	1	18°0 C.	1 min. 45 sec.	
13	2·0	1	18°0 C.	1 min. 20 sec.	
14	2·0	3	16°3 C.	35 sec	Actively swimming for 15–25 seconds
15	2·0	3	16°3 C.	40 sec	
16	3·0	4	16°3 C.	12 sec.	
17	4·0	4	16°3 C.	9 sec.	
18	5·0	3	16°3 C.	5 sec	No recovery
19	8·0	5	16°3 C.	Instantaneous	
20	10·0	5	16°3 C.	Instantaneous	

The action of the sodium chloride depends on the phenomenon of osmosis, the protoplasm being observed to shrink away from the cell wall. In the case of a 1 per cent. solution this contraction takes place and movement ceases. In early experiments in which this occurred it was taken as a sign of death, and it was concluded that a 1 per cent. solution was effective. When the experiment was repeated and the parasites kept under observation for a much longer period, it was seen that they recovered within approximately 30 minutes.

All the concentrations of sodium chloride shown in Table II. are a little too high. The organisms were transferred to the salt solution by means of a fine pipette, and in each case a small drop of water was carried over, thus slightly lowering the concentration of the solution. This small discrepancy would be difficult to avoid, but it does not seriously affect the conclusions. The error is in the right direction, as these experiments were carried out to ascertain the lowest concentration of sodium chloride which would be effective in controlling the parasite

There is a point of interest in reference to tests with the postcystic form; sodium chloride gave the osmotic phenomenon mentioned above, but potassium permanganate, although rapid in its action, produced no visible osmotic change.

The parasite cannot be brought under control while on the fish; control measures must be directed at the three phases of the life cycle of the parasite when it is away from the host. Other workers have attempted control experiments while the parasite was in the pustule, but if the solution used was of a sufficient concentration to destroy the parasite it inevitably led to the death of the fish.

Numerous chemicals have been tried, and some of the reports on their actions have been contradictory. The external application of various chemicals in solution kills and removes those parasites that are about to leave the fish, as well as the smallest forms that have burrowed into the mucous coating. Prytherch (1924) records that experiments with copper sulphate, formaldehyde, sodium bicarbonate, sodium chloride and others, have been successful in killing the parasite, but have so weakened the fish that they either die or soon develop fungus growths and bacterial disease, which eventually prove fatal. He further states that for external treatment of parasitized fish the use of aluminum sulphate (alum sulphate) in solution is most satisfactory. Control work at Ballarat, using sodium chloride and potassium permanganate, has not produced any ill-effects on the fish, although, in the case of the former, the fish were treated for a period ranging from 9 to 14 days. In this case however no attempt is made to reach the parasites while on the fish but to kill them only after they have left the pustules. For this reason a weaker solution may be used and this probably explains the absence of ill-effects to the fish.

Barthélémy (1926) states that tests of many chemicals ($KMnO_4$, NaCl, $HgCl_2$, eau de Javelle, formaldehyde, toluene, chloral hydrate, camphor, pyridine, creosote, H_2O_2, thymol) proved useless, the treated fish dying before the parasite. Also he advises that the contaminated ponds be drained, exposed to sun and heat and that the hands of workmen and the tools be disinfected in milk of lime or 1 per cent. formaldehyde. Ammonia and mercurochrome solutions are suggested for ichthyophthiriasis by Mellen (1926).

Roughley (1933) discusses the use of heat as the most effective treatment for killing the parasite in all stages, including when it is on the fish. This method obviously can only be applied to aquaria. It has been found in America that the parasite will not long survive a temperature of 46° F., and on the other hand, a temperature of from 85° F. to 90° F. also soon proves fatal. On account of the fact that the temperature of the aquarium is more easily raised than lowered, and also because a temperature of

46° F. is a critical one for many goldfish, the higher temperature has been adopted for the treatment of the disease. He then states that a 0.2 per cent. solution of acetic acid in contact with the parasite will kill it almost instantly, but if an infected fish is placed in this solution for several minutes, the parasites atttached to the fish are quite unharmed by it. Similarly he has had an infected fish in a solution containing 25 drops of 2 per cent. mercurochrome to the gallon for three days without the parasites on the fish suffering the slightest harm, although such a solution will invariably kill the parasites with which it is in contact in the course of an hour or two.

Prytherch (1924) and Barthélémy (1926) both considered that the use of a rapid flow of fresh water was the most practical method of combating the parasite. By this means the free parasites are removed as they drop off the fish, the temperature is lowered, the vigour and resistance of the host are augmented and the development of the parasite is diminished.

Fast running water, although perhaps the most satisfactory method of controlling this disease, is not applicable at the Ballarat hatcheries for two reasons: firstly the ponds are connected in series and the parasites would only be driven from one pond to the next; and secondly, all the waste water from the hatcheries is run into Lake Wendouree which is stocked with fish.

Sodium chloride is very satisfactory to use; it is cheap, it is effective in controlling the disease and it does not harm the fish in concentrations sufficient to destroy the parasites in their free living stages. Tests were made at the hatcheries as to the sodium chloride tolerance of trout fry. Time did not permit of a detailed experiment but the following results are of definite value.

TABLE III.—Tests for sodium chloride tolerance of trout fry.

Concentration of NaCl Solution.	Number of Fish.	Length of Experiment.	Temperature.	Remarks.
Per cent.				
3.5	3	1 hr. 23 min.	60° F.	Fish distressed and lying on their sides, but not dead
3.0	3	1 hr. 40 min.	60° F.	Two fish normal. One distressed
2.5	3	1 hr. 38 min	60° F.	Two fish normal. One slightly distressed

Some of the fry at the hatcheries were in poor condition and in the tests using 2.5 % and 3.0% sodium chloride solutions, the fish which showed signs of distress in each case were in a weak condition before the experiments were commenced.

The most serious difficulty met with at the hatcheries was to obtain a distribution of the salt solution throughout the ponds. These are of the following average dimensions: 50 feet long, 15 feet wide at the top, 12 feet wide at the bottom and 4 feet

deep. Each has a capacity of 12,000 gallons. The way in which the difficulty was overcome will be discussed later. The following figures (see Table IV.) give the salt concentrations at various positions in a pond. The pond had been treated with a bag of salt (approximately 180 pounds) per day for some days previously and three bags had been added three hours before the analyses were made. The salt was added by tipping it into the inlet channel so that it went into the pond in solution.

TABLE IV.—Sodium chloride analyses in treated hatchery pond.

Test.	Position in Pond from which the Water Sample was Taken.	Concentration of NaCl.
		Per cent.
1	Inlet end, bottom	1·7
2	Inlet end, 12 inches below the surface	0·3
3	Middle of pond, bottom	2·5
4	Middle of pond, 12 inches below the surface	0·21
5	Outlet end, bottom	3·7
6	Outlet end, 12 inches below the surface	0·18
7	Two ponds beyond, in series with first pond, no individual treatment. Outlet end, bottom	2·4

These figures indicate how the solution falls to the bottom of the pond; the concentration at the bottom increasing and the concentration in the body of the water decreasing as one moves from the inlet to the outlet end of the pond. Analyses were also made in a race which was being treated and the figures were very similar to those given above.

The first outbreak of White Spot at Ballarat was noticed on the 3rd April, 1939. It was not at first diagnosed as White Spot as the fish were heavily infected with Saprolegnia. Salt treatment is also used for Saprolegnia and this was applied. After definite diagnosis of the disease as ichthyophthiriasis more careful measures were undertaken. On 11th May a report was received that the epidemic was abating and early in June the hatcheries were free of the disease. Although the surviving 20,000 yearlings were clean, the Ballarat Fish Acclimatization Society was advised not to liberate these fish in streams in the State. They were held for a time at the hatcheries, and on showing no further signs of the disease, were liberated in Lake Wendouree.

The second outbreak was first observed in the middle of November, 1939, and recurred on three occasions; December 1st, 17th, and 27th, with varying degrees of severity.

Early this season the method of applying salt treatment was to tip 3 bags of salt (approximately 12 bags to the ton) into the pond inlet daily and to leave the inlet pipe open. This would give a salt solution at the bottom of the pond of a concentration as shown in Table IV. $KMnO_4$ was also used in some ponds. A layer of concentrated salt solution at the bottom of the pond was partly effective in that it would kill the forms which had

already encysted, and also the forms dropping down to encyst, but some free-swimming forms would not come into contact with the more concentrated sodium chloride.

A visit was paid to Ballarat in the third week of November and it was realized that the methods previously used were not satisfactory. An attempt was made to combine two methods of control, viz.; the use of sodium chloride treatment and the mechanical means of running water. This was achieved by applying the salt treatment and then cutting off the water for several hours, thus permitting an effective action by the salt solution. The water was then permitted to flow and the second method of control came into force.

This method appeared to have the epidemic under control, but it broke out again on 1st December. The explanation is probably two-fold; firstly, the application of the salt was still inefficient; and, secondly, precautions were lifted too early. A point of great importance in all control work with White Spot is that the treatment must be maintained for a considerable period, for the following reasons. The parasite may be on the fish for a period of from five to fourteen days, this depending on water temperatures, as was stated in the outline of the life cycle of the parasite. Both outbreaks of disease at Ballarat have occurred at temperatures of 60° Fahrenheit or greater. Under such conditions the parasites would probably not stay on the fish for as long as fourteen days—perhaps up to nine days (the times of the appearance of the several outbreaks this season bear this out), although the period would vary. The fish may have been infected on any one of nine successive days so that on applying salt treatment for a short period, only those parasites emerging from the earlier infected fish would be destroyed. The treatment must be maintained over a period of at least ten days and preferably for a longer period if that is practicable.

In an attempt to improve the distribution of the salt solution two modifications in the method of the application of the solution were tried. In the first case the solution was made up in the pump well which supplied the ponds, and in the second, the form of the inlet pipe was altered. The inlet pipe is normally just a little lower than the level of the top of the pond and the water enters in a single jet. An inlet pipe based on a form used in the United States of America was now introduced (see fig. 3). The pipe now went to a depth of 2 to 3 feet into the water and the end of the pipe was plugged. Several holes were bored into the pipe and by this means a greater distribution of the inflowing water was obtained throughout the depth of the pond.

The circulation of the salt solution was still not satisfactory and another outbreak of the disease occurred in different ponds on the 17th December. A further visit was paid to Ballarat and

another attempt was made to surmount the difficulty of circulation. The inlet pipe which had been previously modified was

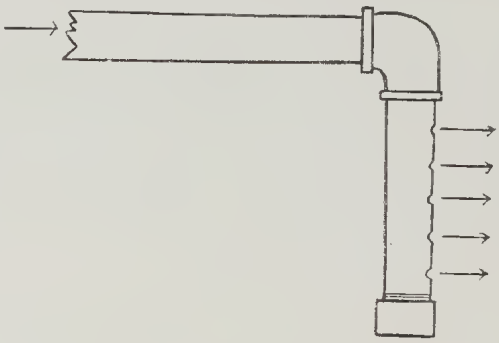

Fig. 3.—Modified form of inlet pipe for hatchery pond.

elaborated still more (see fig. 4). The pipe was lengthened and again holes were bored at intervals. The end was plugged and the pipe was now laid along the bottom over the full length of the pond.

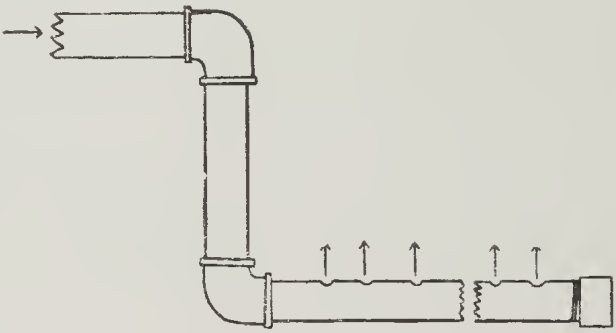

Fig. 4.—Second modified form of inlet pipe for hatchery pond.

Considerable convection currents were obtained by this means and the circulation was appreciably increased. All the infected fish in the hatchery ponds were isolated in a single pond supplied with a pipe of this type. Treatment was first commenced by using 6 bags of salt per day, and this was continued for nine days. The quantity of salt was then reduced to 4 bags per day, and the treatment was carried on for another four days. On the 27th December it was reported that the pond was clean and that there were no further losses. Salt treatment was persisted with and on the 12th January, 1940, the fish were still free of the parasite.

Many of the clean fish, that is fish which had not previously been infected, had been isolated in the hatching boxes. These boxes have no connexion with the ponds and no infection could by any means reach them from the ponds by way of the water system. On the 25th December it was noticed that these fish were infected with White Spot. The only possible explanation is that gear which had been contaminated from infected ponds had been used in the hatching boxes. The warmer weather would give more favorable conditions for the outbreak. These fish also were clean on the 12th January, eighteen days after the infection was first observed, having responded to treatment with sodium chloride. This period is longer than the necessary quarantine period so a favorable conclusion may be drawn. A further report was received from Ballarat on the 23rd January stating that the disease had been brought completely under control, and that the type of pond inlet pipe illustrated in Fig. 4 had proved to be very satisfactory.

As has already been mentioned, sodium chloride is a means of control both for White Spot and a disease caused by the fungus Saprolegnia sp. Saprolegnia played an important role in the White Spot outbreak of the 1938-39 season. As far as is known it cannot attack a healthy fish and there must be some injury for the fungus to become established. The pustules of ichthyophthiriasis supply the necessary injured surface. Following on the first outbreak the fungus was very wide spread and must have been responsible in part for the death of the fish. That the protozoan parasite can itself cause the death of the fish has been well illustrated in the outbreak late in 1939, when the hatcheries were practically free of fungus.

There has been some doubt as to whether the cyst of *Ichthyophthirius multifiliis* can withstand desiccation, and as this is a point of great practical importance, tests were carried out. Cysts were isolated and placed in drops of water and were then allowed to dry off in the air. They were moistened again on two successive days and kept under observation. It was seen that the parasite cannot survive in the absence of water.

Recommendations for the Diagnosis and Treatment of White Spot.

1. If fish are dying in numbers and are showing the symptoms of White Spot, place a few of the dead fish in a tube of water. If the fish are infected with *Ichthyophthirius multifiliis* the parasites will emerge from the pustules within a few hours and can be seen moving around in the water. These parasites are large for Protozoa and can be distinguished macroscopically as

small white roundish objects moving slowly around. This test is applicable to hatcheries where laboratory equipment is generally lacking.

2. Isolate infected ponds by cutting off the water and treat with sodium chloride, as suggested in the discussion on control methods. It is of great importance that the treatment be maintained for a period of from ten to fourteen days, preferably longer if it is possible.

3. After treatment of the infected ponds for this period examine samples of the water at intervals for the free swimming parasites, and if they are still present give further salt treatment. Empty the ponds and allow them to dry out before using again. A further precaution would be to spray the sides and bottom of the empty pond with a concentrated solution of sodium chloride.

4. If waste water from infected ponds does not run into the drainage system but into a lake (as is the case at Ballarat), it must first be treated with sodium chloride. If the waste water leaves the hatchery by way of a race, it is advisable to break down the rate of flow to permit of more effective treatment. This may be done by placing bags in the race, these acting as a baffle.

5. Sterilize all gear such as nets, dipping nets, etc., after use in an infected pond. This may be done by dipping the gear in a concentrated solution of sodium chloride. This precaution prevents the transfer of parasites from one pond to another.

6. Destroy all carp in the hatcheries and sterilize the ponds and aquaria which contained them. In future no carp should be brought into the hatcheries; this statement applies to all fish hatcheries. As far as is known, carp is the original host of the parasite, and in many cases it acts only as a carrier of the disease and suffers no ill-effects.

7. If it is possible, destroy all carp in the vicinity of the hatcheries. In the case of the Ballarat hatcheries this applies to the fish in the adjoining Public Gardens.

8. If the source of the water supply is suspected of carrying infected fish it may be tested in the following way. Pump the water into isolated ponds containing trout fry or yearlings and keep these ponds under observation for the appearance of the disease. If possible, this should be done for a period of fourteen days, and if it is practicable, several ponds should be used, filling them successively at intervals of two days. By this means the period of observation may be extended over a period of from three to four weeks.

9. As the efficiency of the salt treatment depends on the complete circulation of the sodium chloride solution throughout the pond, it is essential that a satisfactory method of water circulation

be devised. As in most cases hatchery ponds are of a considerable size, it is suggested that an inlet pipe of the type illustrated in Fig. 4 be installed. In the absence of disease this type of pipe would still be of service, as the improved water circulation would benefit the fish.

10. It is suggested that a quarantine be applied to all exotic aquarium fish coming into the Commonwealth. The fish should be isolated for a period of three weeks, and if within that time there is no appearance of the disease, they could be declared as free of Ichthyophthiriasis. This measure, while assisting to keep this disease in check, would not greatly interfere with private aquarium owners; valuable fish would still be obtainable, while in all probability the rubbish would be kept out.

Summary.

Outbreaks of White Spot disease of fish (caused by a ciliate protozoan parasite, *Ichthyophthirius multifilis* Fouquet) have occurred at the Ballarat Hatcheries in two successive seasons; yearlings being lost late in the 1938-39 season and fry early in the 1939-40 season.

The life cycle of the parasite is discussed, as it has important bearing on the methods of control.

Conditions favouring an outbreak of the disease in epidemic proportions are considered.

The source of infection is found to be from carp which had been introduced into the hatcheries prior to the first outbreak. These fish had been in contact with Japanese carp which are probably the source of the disease in this country.

Control measures are discussed, the use of sodium chloride being dealt with at length.

Recommendations are made as to treatment of and safeguards against this disease.

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